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The severity and cause of leaf spot disease of *Pongamia pinnata L.* and fungicidal control of the pathogen

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Abstract: A survey on the symptom and severity of the leaf spot disease of *Pongamia pinnata* L. was conducted in the nurseries of the Institute of Forestry and Environmental Sciences, University of Chittagong (IFESCU), Bangladesh Forest Research Institute (BFRI) and Aronnak Nursery in Chittagong. The highest infection percentage and disease index were found in IFESCU nursery, followed by BFRI and the lowest was recorded in Aronnak nursery. The associated organism of leaf spot disease of *P. pinnata* was isolated from the diseased plant parts and the pathogenicity was established with the isolated fungus. *Colletorichum gloeosprioides* Penz was proved to be pathogenic. The inhibition of mycelial growth of *C. gloeosporioides* was observed and identified as suitable fungicides (Bavistin, Cupravite and Dithane M-45) and doses (0.05, 0.10, 0.50, 1.00, 1.50 and 2.00). The lowest and highest mycelial growth were respectively found on Bavistin and on Cupravite at the concentration of 0.05 after 8th day of incubation. It indicates that out of the three tested fungicides, Bavistin showed most effective, followed by Diathane M-45, and Cupravite was ineffective for its very little inhibition on mycelial growth.

Keywords: Severity; Leaf spot; *Pongamia pinnata*; Fungicidal control

Introduction

Pongamia pinnata L. (Known as kerung) belongs to family Fabaceae that is a medium sized, evergreen tree with a short trunk and spreading crown. Different parts of the plants are used for timber, fuel, medicine and industrial purposes (Das and Alam, 2001). Disease is a regular phenomenon in the nursery seedlings and most affected the young plants. Information about different diseases of *P. pinnata* appears very limited. There are records of leaf spot diseases in other countries of the world, except for Bangladesh. Leaf spot diseases recorded mainly involve *Ganoderma lucidum* causing root rot, *Fomes merillii* attacking base of living trees, *Ravelia hobsoni* attacking lower leaf surface and producing chestnut brown teliospore heads with yellowish brown, and also include simple appendages, *R.stictica* and *Microstroma pongamiae* causing leaf coloured spots from white to cream and giving a yellowish appearance to the infected leaves, *Cercospora pongamiae* and *Sphaceloma pongamiae* causing anthracnose spots on leaves, tender shoots and pods resulting in severe damage and early defoliation in young seedlings and trees (Munjal *et al*. 1959; Butler and Bisby 1960; Bakshi and Singh 1967; Browne 1968; Kar and Mandal 1969; Wani and Thirumalchar 1970). The use of fungicides now plays a prominent role in the control of plant diseases (Lima *et al.* 1975; Moline and Locke 1999). These substances are generally applied in liquid or power forms to prevent from the pathogen causing plant diseases and presenting in the air, so that the function of many fungicides are mainly preventive rather than curative. Seedling of *P. pinnata* both in the government and private nurseries in Chittagong are cultivated for plantation. The young seedlings in different nurseries of Chittagong are severely subjected to leaf spot diseases. The present investigation was undertaken to determine the severity of leaf spot diseases of Kerung and the causal organism responsible for the leaf spot diseases in vitro fungicidal control of the fungus causing the disease.

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Materials and methods

Survey and symptoms of leaf spot diseases

Three nurseries namely IFESCU (Institute of Forestry and Environmental Sciences, University of Chittagong), BFRI (Bangladesh Forest Research Institute) and a private nursery (Aronnyak Nursery) in Chittagong Metropolitan area were selected to assess the symptoms and severity of leaf spot diseases of *P. pinnata*. Six-month old seedlings were surveyed in 2003. Random sampling procedure was as Follows: at first three sample plots (each sample plot comprising 100 seedlings) was taken and the total numbers of infected seedling were counted, then from the infected seedlings, ten seedlings were selected at random and total number of leaves as well as infected leaves per seedling was counted. The percentage infected area of leaves was measured through ocular estimation. According to the percentage of infected area, infected leaves were classified into the following five categories. Descriptions of rating were as follows:

Numerical rating	Description of rating
0	Healthy leaves
1	1-10% infected area of leaf
2	11-25% infected area of leaf
3	26-40% infected area of leaf
4	41-60% infected area of leaf
5	>60% infected area of leaf

The severity of diseases in seedlings was determined by using the following formula:

Leaf infection (%)=

$$\frac{\text{Total number of infected leaves}}{\text{Total number of leaves present}} \times 100$$

Diseases index (%) =

$$\frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves per plant} \times \text{Maximum rating}} \times 100$$

The data collected from the infected seedlings in the nursery were recorded individually. Total number of leaves, healthy leaves and infected leaves in a seedling were counted. The symptoms of the leaf spot disease were recorded very carefully in the field condition as well as with detached infected leaves in vitro.

Isolation and identification of the associated organism

Various types of infected leaves were collected from the nurseries for the isolation of associated organism with leaf spot disease from the selected seedlings. Three types of leaves (young, medium-aged and old leaves) were collected. The associated organism was isolated aseptically by placing the surface sterilized (0.01% Mercuric chloride solution) diseased plant tissue on sterilized potato dextrose agar (PDA) medium. The pure culture was prepared in PDA plates and the causal organism was identified. The pure cultures of the fungus were prepared and preserved in the refrigerator to avoid excessive growth and for further study. The fungus isolated from the infected tissues of *P. pinnata* leaves was identified by following the standard keys.

Pathogenicity test

The pathogenicity test was carried out with the isolated fungus both from detached and attached healthy leaves. In pathogenicity test, the leaves were inoculated with mycelial block of 7-8 days old fungus culture (grown in PDA medium) under pricked and unpricked condition. Before inoculation, the detached leaves were slightly injured by needle and the leaves were placed on sterilized filter paper into a tray. On the other hand, the leaves of the seedlings and plants were surface sterilized by washing with ethanol. In all the cases the inoculated leaves were covered with perforated polythene bag. At an interval of 24 h, the inoculated leaves lightly sprayed with sterile water. Observations were made up to 7 days when the leaves of the host plants developed characteristic lesion and then compared with the symptoms recorded during the survey. The pathogen was reisolated from the artificially inoculated leaves following the same procedure as isolation. The morphological characters of the reisolated fungus were compared with the original isolates from the leaves inoculated.

Fungicidal control of the fungus

Effect of different fungicides on mycelial growth was tested on PDA medium. Three fungicides (Bavistin –DF, Cupravite 50wp and Dithane M-45) with different concentrations (0.05, 0.10, 0.50, 1.00, 1.50, and 2.00%) were used to control the pathogen in vitro. Before plating, the fungicides and autoclaved PDA medium were poured into conical flasks firstly, and then mixed thoroughly by shaking the flasks. The prepared medium with definite concentration of fungicide was then taken at the rate of 20 ml per sterilized Petridish. After the solidification of the medium it was inoculated separately with fungal inoculum (5mm dia) of the test fungus. The fungus was taken from the outer margin of the growing cultures on PDA plates. The fungal inocula were then placed at the center of each petridish in an inverted position. The linear radial growth of fungal colony (in mm) was measured in two directions at a right angle to each other after 2 days of incubation. Measurement of fungal colony of *Colletotrichum gloeosporioides* were taken at the 10th. The percentage inhibition of mycelial growth of test fungus was calculated by the following formula:

$$I = \frac{C\text{-}T}{C} \times 100$$

Where, I is the percentage inhibition, C the diameter of fungal colony on PDA, and T the diameter of the fungal colony in treated plates

Statistical analysis: Statistical package such as SPSS 10.OO and DMRT (Duncan Multiple Range Test) are used to analyze the data.

Results and discussion

Symptoms and Severity of leaf spot disease of *P. pinnata*

The leaf symptoms were brownish and more or less circular with dark reddish color surrounding the spot. The enlarged spots were irregular in shape and scattered all over the leaf surface both on the dorsal and on the ventral side. The number of spots varied from one to many in an infected leaf. Spot sizes also varied very widely (Fig. 1). The spots enlarged with maturity of leaves. The mature leaves had higher infection percentage than the young ones.

Fig. 1: Leaf spot of *Pongamia pinnata*

The severity of leaf spot disease of *P. pinnata* was recorded in the three nurseries. It was observed from the Table 1 that the infection percentage and disease index of *P. pinnata* seedlings varied considerably. The highest infection percentage and highest disease index were found in IFESCU nursery, followed by BFRI

and the lowest was recorded in Aronnak nursery. In case of infection percentage, it was observed that the average infection percentage of leaflet was 99.55%, 75.66% and 42.43% in IFESCU, BFRI and Aronnak nursery, respectively. Again, in case of disease index (%) it was observed that the average disease index (%) of leaflet was 82.99%, 59.90% and 15.88% in IFESCU, BFRI and Aronnak nursery, respectively. Nursery disease possesses a great problem in raising disease free healthy seedling on which success of plantation program largely depends. Among the nursery diseases of forest plants leaf spot disease is a regular phenomenon to the younger seedlings. Not much work has been done in identifying and controlling nursery diseases in Bangladesh (Mridha and Fakir 1977; Rahman *et al.* 1982; Basak and Mridha 1987; Rahman 1988; Shayesta 1998; Rahman 1999; Huda *et al.* 2001).

Table 1. Infection percentage and disease index (percentage) of leaf spot disease of *Pongamia pinnata* in three nurseries.

Nursery	Sample plot	Infection percentage/plot	Average infection percentage	Disease index
IFESCU	1	99.66	99.55	81.29
	2	99		88.71
	3	100		78.99
BFRI	1	82.61	75.66	59.52
	2	69.68		54.32
	3	76.19		63.44
Aronnak	1	44.31	42.43	17.83
	2	40.32		14.84
	3	42.64		12.03

The field investigation showed that the disease incidence was severe during dry season, which implied that drought may be one of the important causes. The leaf spot disease was one of the most severe diseases and mainly found in the nursery seedlings during the dry season. At the advent of rainy season the intensity of leaf spot disease becomes less. This indicates that under water stress condition the seedlings might be easily subjected to fungal attack.

Identification and pathogenicity of the fungus

The fungus isolated from the diseased samples was identified as *Colletorichum gloeosporioides*, Penz. The isolated fungus produced typical disease symptoms on leaves of the selected seedlings during pathogenicity test. The sign of infection was observed from the 3rd of incubation. The rate of infection was slow. The highest percentage of infection was observed when pricked leaves were inoculated with mycelial blocks. Very low percentage of infection was obtained from the inoculation of unpricked leaves. The infection of *C. gloeosporioides* was highly depended on leaves age. All types of leaves were found to be susceptible to the fungus. Younger leaflets were more susceptible to *C. gloeosporioides* than the middle aged and mature leaflets.

Leaf spot disease of *P. pinnata* caused by *C. gloeosporioides* is reported probably for the fist time from Bangladesh. The leaf spot of *P. pinnata* caused by *Phyllosticata pongamiae* Syd (Rao, 1964) and *Urohendersomia pongamiae* (Nagraj and Kendrick 1968; Nagraj and Ponappa 1968) was reported from India. *C. gloeosporioides* was also responsible for the cause of different types of leaf spots such as leaf spot of *Alastonia scholaris* (Singh and Katiyar 1969), *Gliricidia sepium* (Amusa and Ezenwa 1996), *Albizia saman*, *Mansonia dipikae* and *Mimusops elengi* (Borah *et al*. 1998), *Amomum dealbatum* (Srivastava and Verma 1999), *Ficus auriculate* (Srivastava and Srivastara 2000). Other that, many researchers (Khan and Hossain 1985; Pal and Purakayastha 1992; Zhang *et al*. 1999) reported *C. gloeosporioides* was a foliar fungus for different plants. These above discussions support that *C. gloeosporioides* has the potentiality to create leaf spot of *P. pinnata*. The present work was compatible with the previous works according to the reports on leaf spot caused by *C. gloeosporioides* of different trees and occurring in several countries.

Effect of different fungicides on mycelial growth of *C. gloeosporioides*

The inhibition of mycelial growth of *C. gloeosporioides*, the causal organism of leaf spot disease of *P. pinnata* was observed to find out suitable agicides and doses of the fungicides. The percent of the increment and inhibition of mycelial growth of the isolated fungus treated with the selected fungicides at different concentrations were presented in table 2, 3 and 4. Out of the three tested fungicides, Cupravite was very ineffective for its very little inhibition on mycelial growth, whereas Bavistin showed the highest effective followed by Diathane –M-45. The growth of the fungus was very slower in Bavistin treated plates as compared to that in other two fungicides. It was also observed that at different concentrations the growth of the fungus was increased with the increase of incubation period. The results of mycelial growth showed that the highest mycelial growth (46.33 mm) was recorded with cupravite at the concentration of 0.05 after 8th day of incubation. The lowest growth (1.70 mm) was found with Bavistin at the concentration of 0.05 on the 8th day of incubation, whereas PDA (control) showed 51.67 mm growth on the same day.

The effect of Bavistin at different concentrations was mentioned in Table 2. It was found that the mycelial growth was very poor at all the experimental concentrations except for control. The growth of the fungus took on an increasing trend with the increase of incubation period at all the selected concentrations. The highest growth was 3.17 mm at a concentration of 1.00 and the lowest was 1.70 mm at a concentration of 0.05. Inhibition percentage was above 90% at all the six concentrations. It was 96.71%, 96.58%, 94.83%, 93.87%, 94.84% and 94.19% at concentrations of 0.05, 0.10, 0.50, 1.00, 1.50 and 2.00, respectively.

The highest growth of mycelium observed in cupravite was 46.33 mm at a concentration of 0.05 (unit) after 8th day of incubation. The lowest growth was 18.00 mm at the concentration of 2.00 from the same fungicide after same day of incubation. The inhibition percentage was 10.33%, 52.91%, 54.19%, 48.38%, 59.36% and 65.16% at concentrations of 0.05, 0.10, 0.50, 1.00, 1.50 and 2.00, respectively.

In case of Dithane M-45, the highest mycelial growth was 33.00 mm at a concentration of 0.10 and the lowest was 4.67 mm in cons. of 1.00 after 8th day of incubation. Inhibition percentage of Dithane M-45 at 0.05, 0.10, 0.50, 1.00, 1.50 and 2.00 concentrations was 43.87%, 36.13%, 55.82%, 90.96%, 72.90% and 90.32%, respectively (Table. 4). Considerable variation in the percent inhibition of mycelial growth was observed in the test fungus due to the fungicidal treatment. It was also observed from the results of the fungicidal control experiments that the highest percent inhibition was produced by Bavistin (96.71%), followed by Dithane M-45 (90.96%) and Cupravite (65.16%) after 8th day of incubation.

Table 2. Effect of Bavistin at different concentrations on the radial growth (mm) and inhibition percentage of mycelium of *C. gloeosporioides*

Name of fungicides	Concentrations (%)	Radial growth of mycelia (mm)						Inhibition (%)
		3rd	4th	5th	6th	7th	8th	
Bavistin	0.05	0.00	0.47	1.00	1.20	1.33	1.70	96.71
	0.10	0.30	0.57	0.95	1.18	1.30	1.77	96.58
	0.50	0.30	0.67	1.40	1.83	2.00	2.67	94.83
	1.00	0.87	1.20	2.20	2.27	3.00	3.17	93.87
	1.50	0.60	0.97	1.63	1.85	2.50	2.67	94.84
	2.00	0.60	1.03	1.70	2.10	2.50	3.00	94.19
PDA (control)		8.44	27.92	36.00	42.33	48.17	51.67	

Table 3. Effect of Cupravite at different concentrations on the radial growth (mm) and inhibition percentage of mycelium of *C. gloeosporioides.*

Name of fungicide	Concentrations (%)	Rate of radial growth of mycelia up to eight days						Inhibition (%)
		3rd	4rd	5rd	6rd	7rd	8rd	
Cupravite	0.05	14.17	21.67	28.00	35.83	39.50	46.33	10.33
	0.10	1.63	10.50	13.33	18.33	23.67	24.33	52.91
	0.50	2.00	9.50	13.17	17.00	20.67	23.67	54.19
	1.00	6.33	10.33	13.33	15.83	22.67	26.67	48.38
	1.50	4.83	10.00	12.83	15.67	19.33	21.00	59.36
	2.00	4.17	7.33	11.00	12.67	15.67	18.00	65.16
PDA (control)		8.44	27.92	36.00	42.33	48.17	51.67	

Table 4. Effect of Dithane M-45 at different concentrations on the radial growth (mm) and inhibition percentage of mycelium of *C. gloeosporioides*

Name of fungicide	Concentrations in (%)	Radial growth of mycelia (cm)						Inhibition (%)
		3rd	4th	5th	6th	7th	8th	
Dithane M-45	0.05	5.20	8.83	11.83	18.50	24.33	29.00	43.87
	0.10	5.77	9.40	14.00	19.33	26.33	33.00	36.13
	0.50	4.80	7.47	10.50	14.50	21.00	22.83	55.82
	1.00	2.67	3.47	4.00	4.47	4.67	4.67	90.96
	1.50	3.00	4.50	6.33	8.67	10.00	14.00	72.90
	2.00	2.30	2.83	3.20	3.63	3.67	5.00	90.32
Control	00	8.44	27.92	36.00	42.33	48.17	51.67	--

Fungicidal control of *C. gloeosporioides* causing different types of leaf spot diseases in many different host plants was studied in various countries **(**Lima *et al.* 1975***;*** Utomo 1987; Moline and Locke 1993; Stirling *et al.* 1999). Utomo in his study on the leaf disease of oil palm nurseries described that Dithane- 45 (mancozeb) spray was effective against *C. gloeosporioides*. Stirling *et al.* (1999) reported that copper fungicide was detrimental to the *C. gloeosporioides* and other microorganism on avocado leaves and fruits. Lima *et al.* (1975) studied the toxic effect of 11 fungicides at three different concentrations on *C. gloeosporioides* and the causal agent of cashew anthracnose. Expect the selected fungicides, Tecto 40F, Daconil 2787, Vitavax, Orthodifolatan 4F and benomyl were found more effective than others. In our study we have also recorded positive results with three different fungicides at different concentrations for the control of *C. gloeosporioides.*

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